IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

TALECRIS BIOTHERAPEUTICS, INC. and BAYER HEALTHCARE LLC, Plaintiffs, v. BAXTER INTERNATIONAL INC. and BAXTER HEALTHCARE CORPORATION, Defendants.	Civil Action No. 05-349-GMS Jury Trial Demanded
BAXTER HEALTHCARE CORPORATION, Counterclaimant, v. TALECRIS BIOTHERAPEUTICS, INC. and BAYER HEALTHCARE LLC, Counterdefendants.	**PUBLIC VERSION**

TRIAL BRIEF OF DEFENDANTS BAXTER INTERNATIONAL INC. AND BAXTER HEALTHCARE CORPORATION

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TABLE OF CONTENTS

TABLE OF AUTHORITIES

Page

Cases

TABLE OF AUTHORITIES

Statutes

Other Authorities

I. INTRODUCTION

Defendants Baxter International Inc. and Baxter Healthcare Corporation (jointly, "Baxter") will prove at trial that Baxter does not infringe any asserted claims of U.S. Patent No. 6,686,191 ("the '191 Patent"). Moreover, the '191 Patent, as interpreted by plaintiffs, Talecris Biotherapeutics, Inc. and Bayer Healthcare LLC (jointly, "Talecris" or "Plaintiffs"), is invalid due to anticipation, obviousness, inadequate written description and indefiniteness. Finally, the patent is unenforceable due to Bayer's inequitable conduct in failing to disclose to the U.S. Patent and Trademark Office (the "PTO") REDACTED and material prior art references.

II. NATURE OF THE CASE

The '191 Patent claims a particular method for making a solution of antibodies essentially free of viruses. Talecris asserts that Baxter infringes the patent by importing into, and selling within, the United States an intravenously-administrable immunoglobulin ("IVIG") solution known as GAMMAGARD Liquid®. Claim 1 is the only independent claim of the '191 Patent; it reads:

> 1. A method of treating a solution of antibodies which may have virus activity, the method comprising
>
> a) contacting the solution with a trialkylphosphate and a detergent under conditions sufficient to substantially reduce any virus activity and resulting in an increased level of anticomplement activity; and
>
> b) then incubating the solution of step a) under conditions of controlled time, pH, temperature, and ionic strength, such that the increased anticomplement activity of the solution is reduced to an acceptable level suitable for intravenous administration.

'191 Patent (attached as Exhibit 1), Col. 11:34-44.

A. The Parties

Baxter, a 75-year-old company, is a leading worldwide manufacturer and supplier of

therapeutic products for the treatment of hemophilia, immune disorders, and kidney disease. Many of Baxter's products are protein therapies derived from blood plasma. Baxter has made and sold immunoglobulin products such as IVIG for more than 20 years. It is an acknowledged pioneer in this industry.

Bayer Healthcare LLC also was a leading supplier of IVIG products in the United States. In 2005, it sold its blood protein business to Talecris Biotherapeutics ("Talecris"), a new company started by two private equity firms. As part of the sale, Talecris received an exclusive license to the '191 Patent. The IVIG product currently sold by Talecris, called Gamunex®, does not practice the invention claimed in the '191 Patent. Nor does Baxter, or anyone else, since the '191 Patent tries to solve a problem that does not exist.

B. The GAMMAGARD Liquid Manufacturing Process

IVIG is derived from blood plasma collected from donors. Since blood plasma collected from human donors may contain viruses, the process of making IVIG incorporates steps to reduce the risk of viral transmission. In addition, when given to patients, IVIG must not trigger an undesirable response of the patient's "complement system" which is a part of the human immune system that responds to potential pathogens in the body. Therefore, IVIG must have a sufficiently low level of anti-complement activity ("ACA") to avoid this response.[1]

Baxter uses a proprietary process to fractionate plasma into its constituent parts.

[1] Even though modern processing techniques substantially reduce the likelihood of severe side effects, patients who receive intravenous injections of IVIG still occasionally experience headaches, fever, chills or other minor "adverse events." Before current manufacturing techniques were developed injections of less refined IVIG sometimes caused severe adverse reactions. Notably, there is no proven correlation between any adverse events and ACA levels.

Baxter's "upstream" fractionation occurs in California and Austria; it is not at issue in this case. Baxter introduced its first IVIG product, GAMMAGARD®, into the U.S. in 1986. Eight years later, the Food and Drug Administration ("FDA") approved Baxter's second generation product, GAMMAGARD® SD. The "downstream" process for making this product included a dedicated solvent/detergent ("S/D") treatment step to eliminate lipid-enveloped viruses. Baxter's S/D process used two detergents, Octoxynol 9 ("Triton") and Polysorbate 80 ("Tween") and one solvent, trialkylphosphate, called "TNBP."[2] Use of this process did not cause any ACA problem, and Baxter's GAMMAGARD SD has been safely administered to patients for more than a decade. Baxter uses this same process in manufacturing GAMMAGARD Liquid, again without experiencing an ACA problem.

In 1998, Baxter International Inc. acquired Immuno, A.G., of Vienna, Austria. At that time, Baxter and Immuno were manufacturing and selling competing lyophilized (powdered) IVIG products. Powdered IVIG is reconstituted as liquid using sterile water.

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Baxter, REDACTED, filed a Biologics License Application with the FDA seeking permission to sell its new liquid IVIG product in the

[2] The basic solvent/detergent treatment step was invented in the 1980s by the New York Blood Center ("NYBC"). Both Talecris and Baxter have licenses from the NYBC permitting the use of this important virus inactivation step.

United States. That application was granted in April, 2005, and sales in the U.S. began in September, 2005.[3]

Baxter's upstream process fractionates blood plasma into a concentrated solution called "Precipitate G." Baxter's downstream process, REDACTED

C. The '191 Patent

Bayer filed the application which led to the '191 Patent on September 22, 1995. Dr. William Alonso is the named inventor. The PTO Examiner rejected all claims as obvious and refused to issue a patent. Bayer appealed, representing to the Board of Patent Appeals that an incubation step was *required* "to reduce ACA to an acceptable level for IV administration" due to an "unexpected" increase in ACA caused by S/D treatment. Relying on this assertion, the claims were allowed. D.I. 161 at JA99. In fact, however, there was no invention; the increased ACA was purely a result of the suboptimal S/D process used by Bayer.

In performing the experiments on which it based this patent, Bayer used a harsh detergent – sodium cholate – that is different from the detergents used by Baxter. *See* Ex. 1 ('191 Patent), Col. 4:13-29; Col. 6:18-Col. 9:55. It applied this solvent and detergent mix for

[3] REDACTED

ten hours. Ex. 1 ('191 Patent), Col. 6:35-39; Col. 7:24-26. Moreover, the experiments upon which Claim 1 is based were run at pH 7.0. This is not an ideal pH for virus inactivation, as the inventor himself acknowledged. *Id.*, Col. 4:4-29; Col. 8:6-36. Under these extreme conditions, Bayer observed that ACA was higher than when these particular, and harsh, conditions for solvent/detergent treatment were not used. Overgeneralizing, Bayer broadly asserted that "[t]he ACA increase resulting from the solvent/detergent treatment of the IGIV (antibody) solution appears to be an **unavoidable secondary effect** of the TNBP/detergent treatment to inactivate viruses in a solution." *Id.*, Col. 9:58-61 (emphasis added). The observed ACA "increase" was measured using a "hemolytic" assay (test) that is described generally in the '191 Patent. *See id.*, Col. 5:56-Col. 6:16.

Because it used such extreme conditions for its solvent/detergent treatment, Bayer created its own problem – elevated ACA – which it then purported to solve: "I have discovered that by incubating the solution of IGIV at a low pH (4.25) and low ionic strength (0.001M) for a relatively long period of time (at least about ten days), the ACA gradually decreases over the period of incubation." *Id.*, Col. 9:61-65. But when different, less harsh (and more effective) solvent/detergent conditions are used, ACA does not increase. Baxter uses a different solvent/detergent combination, REDACTED

As a result, ACA does not increase after Baxter's solvent/detergent treatment. Nevertheless, Talecris contends that Baxter's process, if it were performed in the U.S., would infringe Claims 1, 7 - 10, 12, and 15 - 20, thus violating (allegedly) 35 U.S.C. § 271(g).

III. THE CONTESTED FACTS TO BE PROVEN AT TRIAL

A. Baxter Does Not Infringe

Tests conducted by both Talecris and Baxter prove that Baxter's GAMMAGARD Liquid process does not infringe the '191 Patent. REDACTED

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REDACTED[4]

REDACTED[5]

REDACTED[6]

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This consistent evidence from both parties' testing of three different lots of GAMMAGARD Liquid, using the same type of ACA test the inventor used in the '191 Patent, proves Baxter

[4] "As used herein, one unit of ACA activity (one CH_{50} unit) is defined as the amount of protein capable of activating 50% of the complement in an optimally titered complement and **red blood cell/hemolysin** system." Ex. 1 ('191 Patent), Col. 5:64 - Col. 6:1 (emphasis added). The hemolytic assay is generally described in Col. 6:1-16.

[5] REDACTED

[6] The units of measure in ACA assays vary from test to test, and test results from different tests are not comparable. Thus, the relevant question at this stage is whether ACA levels increased or decreased using the units of measure in the particular assay.

does not infringe the '191 Patent. [7]

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Thus,

again, Baxter does not infringe.

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[7] Parallel litigation between the parties is pending in Belgium where Baxter manufactures GAMMAGARD Liquid. In that proceeding, the Court has appointed an independent technical expert to evaluate the claim of infringement. The parties submitted to that expert the expert reports submitted in this case by Drs. Carroll and Ravetch, for Plaintiffs, and Dr. Terence Snape, for Baxter. On April 23, 2007, that independent expert concluded that the Snape testimony – there is no ACA increase caused by Baxter's solvent/detergent treatment – is correct.

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In addition to not meeting the elements of Claim 1, the GAMMAGARD Liquid process also differs from the asserted dependent claims in additional ways.

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Finally, Baxter's S/D process uses two detergents, including Octoxynol 9, which is not included in the Markush Group of Claim 19.

B. The '191 Patent Is Invalid

The evidence also will prove the '191 Patent is invalid for indefiniteness and inadequate written description. The patent never specifies what is meant by an "acceptable level [of ACA] suitable for intravenous administration," or a particular hemolytic assay that should be used to measure ACA. Nor is there any industry-wide understanding of the term, "acceptable level".

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Consequently, "acceptable level" lacks written description as well.

Bayer represented to the PTO that solvent/detergent treatment "inherently" increases ACA. If that is true, then the '191 Patent is invalid for anticipation since prior art already used an S/D treatment followed by an incubation step when processing immunoglobulin therapies. *See* Ex. 6. In any event, a manufacturing process that included incubation after solvent/detergent treatment is obvious. Both processing steps already were well known in the art, and reducing virus activity was a constant goal of IVIG producers.[8] *See* Exs. 6 and 7.

C. Bayer's Inequitable Conduct

Its representations to the PTO notwithstanding, Bayer actually knew that solvent/detergent treatment did not *always* increase ACA.

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This is inequitable conduct that renders the patent unenforceable.[9]

[8] REDACTED.

[9] Baxter currently has a motion pending before the Court to permit Baxter to assert the defense of inequitable conduct at trial, based upon these facts. *See* D.I. 166.

D. No Willful Infringement

Finally, in the unlikely event Talecris succeeds in proving infringement and upholding the validity of the '191 Patent, the evidence will prove Baxter did not infringe willfully. Baxter has used the same solvent/detergent step for fifteen years and has never had an ACA problem with that step. Moreover, REDACTED

Thus, Baxter has always had a good faith belief it does not infringe the '191 Patent.

E. Damages

Talecris concedes it cannot recover damages for lost profits due to Baxter's alleged infringement REDACTED

Indeed, all suppliers of this much-needed therapeutic are selling at maximum capacity. Thus, even if the jury finds for Talecris, the Court should allow Baxter to continue to sell GAMMAGARD Liquid because it is needed by patients and there are no other available sources.

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IV. THE APPLICABLE LAW

To prove infringement, Talecris must prove by a preponderance of the evidence that Baxter's GAMMAGARD Liquid manufacturing process utilizes every element of at least one asserted claim of the '191 Patent, literally or by a substantial equivalent. *Laitram Corp. v. Rexnord, Inc.*, 939 F.2d 1533, 1535 (Fed. Cir. 1991). Proof of infringement under the Doctrine of Equivalents requires that ***individual elements*** of the accused process perform

"substantially the same function in substantially the same way to obtain the same result" ("function-way-result"). *Graver Tank & Mfg. Co. v. Linde Air Prods. Co.*, 172 U.S. 605, 608 (1950). Alternatively, Talecris must show there are only insubstantial differences between each element of the claims and the accused process. *Warner-Jenkinson Co., Inc. v. Hilton Davis Chem. Co.*, 520 U.S. 17, 39-40 (1997). A dependent claim cannot be infringed unless the independent claim upon which it depends also is infringed. ROBERT HARMON, PATENTS AND THE FEDERAL CIRCUIT, § 7.2(a)(i), pg. 381 (7th ed., BNA, 2005); *Wolverine World Wide, Inc. v. Nike, Inc.*, 38 F.3d 1192, 1199 (Fed. Cir. 1994).

To prove the '191 Patent is invalid, Baxter must introduce clear and convincing evidence. *Minnesota Mining and Mfg. Co. v. Chemque, Inc.*, 303 F.3d 1294, 1301 (Fed. Cir. 2002); *McGinley v. Franklin Sports, Inc.*, 262 F.3d 1339, 1349 (Fed. Cir. 2001). Baxter contends the claims of the patent are anticipated and/or obvious, that the claims are indefinite, and that the patent fails to provide an adequate written description of the claimed invention.

Baxter currently has a summary judgment motion pending which asserts that the undisputed facts prove the claims of the '191 Patent are insolubly indefinite. Title 35 of the United States Code, Section 112, requires that a patent's specification, "… conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention." 35 U.S.C. § 112, ¶ 2. "[T]he purpose of the definiteness requirement is to ensure that the claims delineate the scope of the invention using language that adequately notifies the public of the patentee's right to exclude." *Datamize, LLC v. Plumtree Software, Inc.*, 417 F.3d 1342, 1347 (Fed. Cir. 2005). The definiteness requirement also insures that "the claims are written in such a way that they give notice to the public of the extent of the legal protection afforded by the patent, so that interested members of the public,

e.g., competitors of the patent owner, can determine whether or not they infringe." *All Dental Prodx, LLC v. Advantage Dental Prods., Inc.*, 309 F.3d 774, 779-80 (Fed. Cir. 2002); *accord Oakley, Inc. v. Sunglass Hut Int'l*, 316 F.3d 1331, 1340 (Fed. Cir. 2003).

Baxter contends the claim terms "acceptable level suitable for intravenous administration," "increased level of anticomplement activity," "then incubating the solution of step (a)," "reduced to an acceptable level," and "the increased anticomplement activity of the solution" all are indefinite. "[A] claim is indefinite under § 112 ¶ 2 if it is 'insolubly ambiguous, and no narrowing construction can properly be adopted.'" *Amgen Inc. v. Hoechst Marion Roussel, Inc.*, 314 F.3d 1313, 1342 (Fed. Cir. 2003) (citations omitted).

In *Honeywell Int'l, Inc. v. Int'l Trade Comm'n*, 341 F.3d 1332, 1335 (Fed. Cir. 2003), the court invalidated a patent due to indefiniteness under facts quite similar to those present here. The invention in *Honeywell* was for producing synthetic yarn. The last claim term recited "thereby obtaining a drawn yarn with a terminal modulus of at least 20 g/d and a melting point elevation [MPE] of 10 C. to 14 C." The method used for measuring MPE was not recited in the claims, yet three different methods of making the measurement existed and each provided different MPE numbers. *Id.* at 1335-36, 1339. Under these circumstances the Federal Circuit held the claim "insolubly ambiguous, and hence indefinite .... [T]he claims, the written description, and the prosecution history fail to give us, as the interpreter of the claim term, any guidance as to what one of ordinary skill in the art would interpret the claim to require." *Id.* at 1340; *see also Datamize*, 417 F.3d at 1352 ("[r]eference to undefined standards, regardless of whose views might influence the formation of those standards, fails to provide any direction to one skilled in the art attempting to determine the scope of the claimed invention."); *Halliburton Energy Servs., Inc. v. M-I, LLC.*, 456 F. Supp. 2d 811, 820 (E.D.

Tex. 2006) (claims invalidated because no objective standard was provided for the claim term "fragile gel." "Although 'a patentee need not define his invention with mathematical precision to satisfy the definiteness requirement,' there must be some 'objective anchor' by which skilled artisans can identify whether they are practicing the patented invention.")

Baxter contends that each of the asserted claims is invalid because the specification of the '191 Patent is not sufficiently clear to allow a person of ordinary skill in the art to immediately discern the invention as finally claimed in the patent. *See Univ. of Rochester v. G.D. Searle & Co.*, 358 F.3d 916 (Fed. Cir. 2004); *TurboCare Div. of Demag Delaval Turbomachinery Corp. v. Gen. Elec. Co.*, 264 F.3d 1111, 1118 (Fed. Cir. 2001). In particular, Baxter contends that all asserted claims lack adequate written description of the terms "trialkylphosphate and a detergent," "under conditions sufficient to substantially reduce any virus activity," "conditions … resulting in an increased level of anticomplement activity," "the increased activity of the solution" and "reduced to an acceptable level suitable for intravenous administration." Baxter also contends, if "acceptable level suitable for intravenous administration" is defined by release limits or adverse events, that all asserted claims of the '191 Patent are invalid as lacking adequate written description of the term "acceptable level suitable for intravenous administration." Baxter also asserts that Claim 9 is further invalid as lacking an adequate written description of "wherein the temperature is maintained within a range of 2°C to 50°C."

Baxter contends each asserted claim is invalid as anticipated by prior public knowledge. *See* 35 U.S.C. §§ 102(a), 102(b). A list of the anticipatory prior art Baxter will rely upon at trial is attached as Exhibit 6. Baxter also will prove all asserted claims are obvious, particularly since there was a need in the market for an IVIG product with as little

virus contamination as possible, and there were a finite number of identified, predictable solutions. *See* 35 U.S.C. § 103; *KSR Int'l Co. v. Teleflex, Inc.*, 127 S. Ct. 1727 (April 30, 2007); *Pfizer, Inc. v. Apotex, Inc.*, 82 U.S.P.Q.2d 1321, 1331-33 (Fed. Cir. 2007). A list of the additional prior art Baxter will rely upon to establish the claims of the '191 Patent were obvious is attached as Exhibit 7.

Baxter has pending a motion to be permitted to add an allegation that the '191 Patent is unenforceable due to inequitable conduct. REDACTED

That motion is currently pending. *See* D.I. 166. At trial, Baxter would have to prove inequitable conduct by clear and convincing evidence. *Bristol-Myers Squibb Co., v. Rhone-Poulenc Rohrer, Inc.*, 326 F.3d 1226, 1233-34 (Fed. Cir. 2003).

V. REMEDIES

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This unprecedented alternative damage analysis is unauthorized by any applicable law and asks the jury to do the impossible: render a verdict based upon a guess as to whether the Court will or will not enjoin the sale of GAMMAGARD Liquid. Because Plaintiffs' "alternative" damage model is improper, Baxter has filed a motion *in limine* to exclude it.

In any event, both of the royalty rates sought by Plaintiffs are ridiculously high. Industry-standard rates for improvements in processing are substantially lower.

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VI. ANTICIPATED MOTION FOR A DIRECTED VERDICT

Baxter believes it will be entitled to a directed verdict of non-infringement because the test results discussed in Section III.A, *supra*, reveal that ACA does not increase as a result of Baxter's solvent/detergent treatment, and certainly does not increase to an unacceptable level. With these results, Talecris cannot meet its burden of proof. In addition, Baxter believes the Court will decide as a matter of law that the patent claims are invalid for indefiniteness and lack of adequate written description and, therefore, should direct a verdict that the asserted claims are invalid.

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EXHIBIT 1

US006686191B1

(12) **United States Patent**
Alonso

(10) **Patent No.: US 6,686,191 B1**
(45) **Date of Patent: Feb. 3, 2004**

(54) **PREPARATION OF VIRALLY INACTIVATED INTRAVENOUSLY INJECTABLE IMMUNE SERUM GLOBULIN**

(75) Inventor: **William R. Alonso**, Cary, NC (US)

(73) Assignee: **Bayer HealthCare LLC**, Tarrytown, NY (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 1772 days.

(21) Appl. No.: **08/532,211**

(22) Filed: **Sep. 22, 1995**

(51) **Int. Cl.**[7] **C12N 7/04**; A61K 39/395; A61K 39/40; A61K 39/42

(52) **U.S. Cl.** **435/236**; 424/176.1; 424/177.1; 424/130.1

(58) **Field of Search** 530/390.1, 390.5, 530/386, 387.1; 424/176.1, 177.1, 130.1; 435/236

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,396,608 A * 8/1983 Tenold
4,540,573 A * 9/1985 Neurath et al.
4,762,714 A * 8/1988 Mitra et al.

OTHER PUBLICATIONS

Joy Yang, Y.H. et al. "Antibody Fc functional activity of intravenous immunoglobulin preparations treated with solvent–detergent for virus inactivation" Vox Sang, vol. 67, pp. 337–344, May 17, 1994).*

* cited by examiner

Primary Examiner—Yvonne Eyler
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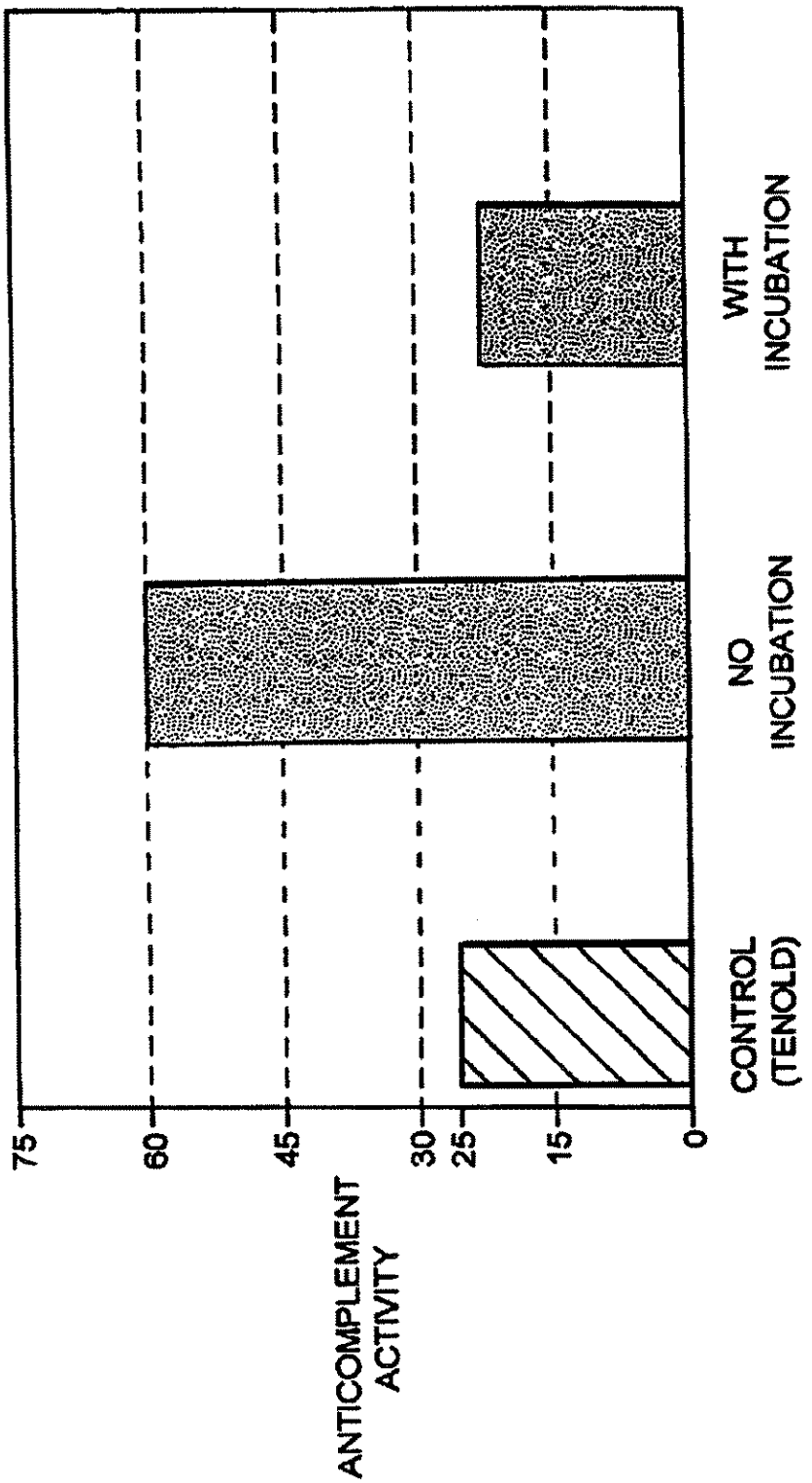
(57) **ABSTRACT**

Method of reducing the anticomplement activity (ACA) resulting from viral inactivation treatment of a solution of antibodies, the method comprising contacting the solution with a trialkylphosphate, such as tri-n-butyl phosphate, and a detergent, such as sodium cholate, under conditions sufficient to reduce substantially the virus activity, and then incubating the solution under controlled conditions of time, pH, temperature, and ionic strength such that the anticomplement activity is reduced to an acceptable level. In a preferred embodiment, the ACA is reduced to less than 60 CH_{50} units/mL, the incubation is for at least about ten days at a pH from 3.5 to 5.0, the temperature is maintained within a range of 2 to 50° C., and the ionic strength of the solution is less than about 0.001 M.

24 Claims, 1 Drawing Sheet


ANTICOMPLEMENT ACTIVITY
75
60
45
30
25
15
0
CONTROL (TENOLD)
NO INCUBATION
WITH INCUBATION

PREPARATION OF VIRALLY INACTIVATED INTRAVENOUSLY INJECTABLE IMMUNE SERUM GLOBULIN

BACKGROUND OF THE INVENTION

1. Field

This invention generally deals with an intravenously injectable immunoglobulin product, and more specifically deals with an intravenously injectable immune serum globulin (IGIV) which has been subjected to a virus inactivation step and which has a low level of anticomplement activity.

2. Background

Early pharmaceutical preparations of immune serum globulins could not be administered intravenously due to an unacceptably high incidence of adverse reactions. These adverse reactions were associated with a decrease in serum complement levels, apparently caused by complement binding to the administered gamma globulin. (1) The ability of gamma globulin to bind complement, or its anticomplement activity (ACA), is greatly increased as a result of denaturation brought about during the fractionation procedure. Several approaches have been taken to address the problem of rendering ISG safe for intravenous administration. (See (2) and references therein). Tenold reported a method of preparing an immune serum globulin (ISG) with low ACA which could be administered by intravenous injection. (2, incorporated herein by reference). The Tenold '608 process requires formulating the ISG at low ionic strength (preferably less than about 0.001) and at low pH (3.5–5.0).

Other methods of preparing intravenously injectable immune serum globulin (IGIV) have been reported, including stabilizing with carbohydrates such as maltose (3). A process including incubation of ISG at pH 4.0 at 37° C. (4) results in a product with low ACA which may be administered by intravenous injection; however, upon storage the product regains its high ACA. IGIV has also been prepared by covalent modification of the ISG, for example by proteolysis (5) or by reduction of disulfide linkages followed by reaction with a blocking agent (1,6).

Antibody preparations, since they are isolated blood products, have an inherent hazard of transmitting virally-mediated diseases. Inactivation of viruses is an important step in producing safe and effective blood products. U.S. Pat. No. 4,540,573 to Neurath et al., which is incorporated herein by reference, describes a viral inactivation process using a trialkyl phosphate and detergent process (hereinafter, the solvent/detergent process, or SD process). (7) That solvent/detergent method has gained acceptance as being efficacious in the inactivation of lipid-enveloped viruses with limited adverse effects on biological activity or blood product profile. (8, 15; See also 12 for a discussion of various viral inactivation processes).

Current antibody preparations on the market generally have been regarded as safe with respect to viral contamination. (9) This is thought to be due to features of the fractionation processes used to isolate these blood products. However, it would be desirable to further ensure the safety of the antibody preparations by including a distinct viral inactivation step in the production process. Successful reduction of viral activity in an IGIV solution was reported using several different methods of viral inactivation for a variety of viruses. (16, 17) A process for preparation of immunoglobulins substantially free of retrovirus has been reported involving incubation of ISG under controlled conditions of time, temperature, and pH. The process entails

isolating ISG via a cold ethanol plasma fractionation process and then storage of the ISG at one of two storage conditions: (a) at pH≦4.25 at a temperature of 27° C. for at least three days, or (b) at pH≦6.8 at a temperature of 45° C. for at least six hours. (10).

We have found that using the SD process to treat ISG preparations, especially those subsequently formulated according to the Tenold '608 patent, results in a product with an acceptable viral inactivation but with unacceptably high levels of ACA. Elevated ACA levels were always detected at the sterile bulk stage (i.e., after compounding as 5% or 10% IGIV and filtration with 0.2 μm sterile filters) of all tri-n-butyl phosphate (TNBP)/detergent treated IGIV preparations regardless of process scale. Preparations of ISG with high ACA levels are not suitable for intravenous injection and instead must be administered via other routes, e.g. intramuscular (IM) injection. However, IGIV preparations are more desirable since they are immediately available in the bloodstream and are not subject to loss associated with IM injection. It is thus desirable to have an IGIV product which is both low in ACA and has been subjected to a viral inactivation step.

SUMMARY OF THE INVENTION

The invention is a method for producing an intravenously injectable immune serum globulin (IGIV) preparation with low anticomplement activity which has been chemically treated to render it substantially free of lipid-enveloped viruses. The method comprises a solvent/detergent viral inactivation step followed by an incubation step. We have discovered that the incubation step is necessary to achieve an acceptable level of ACA low enough to allow the ISG to be administered by intravenous injection. The incubation step should be conducted under controlled time, pH, temperature, and ionic strength. Preferably, the pH should be maintained between about 3.5 and about 5.0, the temperature should be within a range of about 2 to about 50° C., and the ionic strength should be less than about 0.001M. In a preferred embodiment the ACA of the ISG preparation decreases gradually over a period of at least about ten days when the ISG is maintained at a pH of about 4.25 at low ionic strength (less than about 0.001M) and the viral inactivation step (in a model system) results in a substantial reduction (i.e. at least 4 logs) in the titer of lipid enveloped viruses.

BRIEF DESCRIPTION OF THE FIGURE

FIG. 1 shows a comparison of the typical average observed ACA levels of 5% IGIV solutions treated according to the SD process and with or without the follow-up incubation of the present invention.

SPECIFIC EMBODIMENTS

Materials and Methods

The starting material for the process of this invention is unmodified human immune serum globulin. In the specification and claims the term "immune serum globulin" is used to define the substance also referred to in the literature variously as gamma globulin, IgG and immunoglobulin G. It consists predominantly and preferably of at least about 85 percent of the 7S species of gamma globulin, which has a molecular weight of about 160,000. Any remainder is preferably 9S species, with a molecular weight of about 300,000. Both standard immune and hyperimmune serum globulins, e.g., tetanus, rabies and hepatitis immune serum globulins,

can be employed, the solvent/detergent treated product being immune and hyperimmune ISG, respectively. Thus, a suitable starting material for the process of this invention is Cohn's Fraction II or Fraction III filtrate. (See Refs. 13, 14.)

Fraction II, by ultracentrifugation studies, is predominantly (about 85 percent) the 7S (sedimentation constant of 7) species of gamma globulin with an average molecular weight of 160,000. The remaining protein is essentially 9S material with a M.W. of about 300,000. Wet Fraction II paste (approximately 30 percent solids) is commonly lyophilized to obtain dry ISG powder which is then dissolved and prepared for intramuscular injection as a 16.5 percent sterile solution. Either the wet Fraction II paste or the dry ISG powder is a suitable starting material for the process of this invention.

Gamma globulin obtained by any process which has essentially the same composition of protein components as found in the Cohn Fraction II or Fraction III filtrate can be used as starting material in the present process. Both standard immune serum globulin and hyperimmune serum globulin can be employed as starting materials. As is well known, the latter is produced from plasma or serum obtained from selected donors who have much higher titers for a specific antibody than is normally found in the average population. These donors have either been recently immunized with a particular vaccine or else they have recently recovered from an infection or disease. These high titer sera or plasmas are pooled and subjected to the usual Cohn fractionation procedures up to the point of isolating Fraction II.

Furthermore, because the amount of antibody required to achieve a desired immunological response is substantially less when administered intravenously, it will be apparent the intravenous dose will be substantially less than the intramuscular dose which will produce the same serum antibody titer. Thus, the dose of intramuscular ISG and hyperimmune serum globulin must be higher than that required to achieve the same serum antibody titer when globulin of the same antibody activity is administered intravenously.

The starting wet paste or lyophilized powder is dissolved in a volume of water or other physiologically-acceptable carrier to provide a protein solution of a concentration of about 0.5–20% preferably about 5 to 10 percent. If Fraction III filtrate is employed, the aqueous solution must be concentrated by conventional techniques to the desired protein concentration. Any protein concentration may be used in this method; however, the above range is preferred from a practical standpoint.

After the protein has been dissolved or concentrated, the solution is adjusted to a pH of about 3.5 to 5.0 preferably about 3.8 to 4.2, by addition of a physiologically-acceptable acid such as hydrochloric acid. In general, the pH is adjusted to a point whereat the monomeric material in the protein solution is maintained at a maximum. However, the pH must not be so low as to result in gelation. The temperature should not be harmful to the ISG material. Good results are obtained within the temperature range of about 0–20° C. It is not necessary to hold the so-adjusted material for any period of time prior to the next step; however, the material may be held, if desired, without detrimental effects.

The protein solution at the appropriate pH (preferably 3.8–4.2) may be diafiltered with at least 4 volume exchanges of water to reduce the alcohol concentration from approximately 17% (Filtrate III) to about 2% alcohol. The efficacy of solvent/detergent as a viral inactivation method is much better at or above ambient temperatures; however, high concentrations of alcohol at these temperatures will denature the IgG molecules. Thus, this inactivation must be performed in low alcohol concentration.

Next, the protein concentration of the so-treated material is adjusted to the level desired for incubation with TNBP/detergent, generally less than 10% protein for maximum viral inactivation. This adjustment is accomplished by conventional techniques not detrimental to ISG, e.g., ultrafiltration, reverse osmosis, sublimation, evaporation, etc. Prior to addition of TNBP/detergent, the pH may be adjusted within a wide range, depending on the detergent to be used. With Tween 80, the pH may be as low as 3.5, where the IgG starts becoming unstable. With cholate, the pH is adjusted to within the range of 5.0–6.4, preferably about 5.6, prior to addition of TNBP/detergent. Satisfactory cholate solubility during incubation was achieved by adjusting the immunoglobulin solutions to a pH of 5.5 or higher prior to addition of TNBP and sodium cholate. Adjusting the IgG solution to pH values lower than 5.5 is not suitable because the solubility of sodium cholate is highly dependent on pH (cholic acid pK=6.4), with poor solubility at pH 5.5 or lower. Furthermore, maximum viral inactivation during incubation with TNBP/cholate was observed at pH values less than 6.0 in experiments which employed model viruses spiked into IgG solutions. The inactivation of HIV-1 and BVDV (bovine viral diarrhea virus, which is employed as a model for hepatitis C) was accelerated at pH 5.8, with inactivation to the detection limit occurring in 1–2 hours, whereas inactivation to the detection limit required a minimum of 6 hours when pH 7 conditions were used.

Next, the TNBP/detergent is added to the protein solution (preferably less than 8% [w/w], pH 5.8) mixed thoroughly, and then incubated above ambient temperatures, for example 30° C., with continuous agitation or mixing. Target TNBP/cholate levels for optimal viral inactivation during the incubation step should be >3 mg/mL TNBP and >2 mg/mL cholate as defined by Edwards et al. (8) Moreover, for effective viral inactivation, it is important that the solution is essentially free of particulates in order to facilitate thorough mixing of solvent/detergent and IgG solution. After incubation with TNBP/cholate under these conditions, greater than 5.2 $\log_{10}$ reduction of HIV-1 and greater than 4.0 $\log_{10}$ reduction of BVDV were detected.

After completing the incubation which provides the viral inactivation, the solvent and detergent molecules must be removed in order to achieve a final product with low levels of residual TNBP and cholate which would be suitable for intravenous administration. Generally, procedures to remove detergent are also effective in removing TNBP, and vice versa. Very low levels of TNBP and cholate in the final container can be achieved by a combination of filtration, diafiltration and hydrophobic chromatography. After completing the incubation, the majority of cholate (and TNBP) can be removed from the protein solution by filtration, providing the solution had been previously adjusted to a lower pH value such as 4.0, because sodium cholate is sparingly soluble in aqueous solutions at such pH values. Moreover, all processing steps which follow the solvent/detergent incubation are performed at lower pH values (i.e., 4.0) because IgG molecules are more stable at pH values between 3.5–5.0, in low ionic strength solutions. (2) Thus, after incubation with TNBP/cholate, the protein solution is adjusted to approximately pH 4.0 and incubated at 0–8° C. in order to promote cholate precipitation. Next, filtration is employed to remove the precipitated cholate from the IgG solution.

The so-treated solution is diafiltered with at least four volume exchanges of water to reduce the ionic strength and

to remove additional TNBP and cholate. After or during the above treatment, the pH is measured and maintained within the range of about 3.5–5.0. The protein concentration of the so-treated material is adjusted to 10–30%, usually 13% (w/v) by employing conventional techniques not detrimental to ISG, e.g., ultrafiltration, reverse osmosis, sublimation, evaporation, etc. Again the pH of the preparation is maintained within the range of about 3.5–5.0, preferably about 3.8–4.2.

In the present invention, hydrophobic chromatography is employed to remove the TNBP and cholate not eliminated by the filtration and diafiltration steps, and thus provide a final product with low levels of residual TNBP and cholate which is suitable for intravenous administration. Hydrophobic chromatography is a method for TNBP removal from protein solutions that has fewer drawbacks and limitations than other available methods such as oil extraction, ion exchange or affinity chromatography. In part, this is because the protein of interest (IgG) remains in solution throughout the TNBP removal process. Polystyrene-based resins (typically PLRP-S from Polymer Laboratories, Amherst, Mass.) were used to remove the solvent/detergent from solution, as we have found the polystyrene-based resins to be superior to other resins, such as silica-based C-18 resins.

Next, the ISG preparation is adjusted to 5% or 10% protein, and treated to render it tonic, i.e., to render it compatible with physiological conditions, or render it physiologically acceptable upon injection. In a preferred embodiment, the tonicity is adjusted to about 230 to about 490 mosmol/kg solvent. More preferably, the tonicity range is from about 250 to about 350 mosmol/kg solvent, and most preferably the tonicity range is from about 260 to about 325 mosmol/kg solvent. The 5% formulation (5% IGIV) is made tonic by the addition of 10% maltose. The 10% formulation contains 0.2 M glycine in order to achieve an isotonic preparation without large quantities of sugar. The product with either formulation (Gamimune®N 5% or Gamimune®N 10%) experiences shifts in molecular distribution (antibody aggregation) when the ionic strength of the low pH solution is increased. Therefore, sodium chloride, which is often used to achieve tonicity, should not be used.

The so-treated solution is incubated at pH 4.25 under low ionic strength conditions (NLT 21 days at 20–27° C. preferred) in order to provide a lowering of ACA levels. The ionic strength is determined according to Perrin (18), and in a preferred embodiment the ionic strength should be less than about 0.001M. Elevated ACA levels were always detected at this stage of all TNBP/cholate treated IGIV preparations (regardless of process scale); however, ACA levels are gradually lowered by incubation at pH 4.25 under low ionic strength conditions (Tables 3, 5–7). While there is no strict rule for determining when the ACA level is low enough to be an acceptable level suitable for intravenous administration, IGIV preparations should have ACA levels as low as possible.

The Figure depicts the typical average reduction of ACA observed in 5% IGIV solutions following SD treatment. For a 5% ISG formulation the acceptable level suitable for intravenous administration preferably would be less than about 45 CH_{50} units/mL, and more preferably less than about 30 CH_{50} units/mL. For a 10% ISG formulation, the acceptable level suitable for intravenous administration preferably would be less than about 60 CH_{50} units/mL, and more preferably less than about 45 CH_{50} units/mL. As used herein, one unit of ACA activity (one CH_{50} unit) is defined as the amount of protein capable of activating 50% of the complement in an optimally titered complement and red blood cell/hemolysin system. The assay measures the amount of complement that is bound by the mixture of standardized amounts of complement and protein. See refs. 19–20 for a discussion of the assay. Briefly, red blood cells that have been sensitized by preincubation with red blood cell antibodies are added to the complement/protein mixture. In the presence of free complement (not already bound by the protein) these sensitized cells will lyse, releasing hemoglobin which can be quantitated as a measure of the degree of lysis. In parallel, sensitized red blood cells are also added to a buffer control-complement mixture, whose degree of lysis is defined as 100%. The difference between the actual amount of complement needed to give 100% lysis and the amount of complement remaining unbound in the presence of protein equals the amount of complement actually bound by the protein, or anticomplement activity.

Results

Anticomplement Activity of ISG Resulting From Viral Inactivation Process

To establish the effect of the SD viral inactivation process on solutions containing ISG which are formulated according to the Tenold '608 patent, the experiments depicted in Table 1 were performed. The starting material (SM) was Cohn process filtrate III which had been ultrafiltered to about 5% protein and then diafiltered with four volumes of water.

In the control experiment, incubation (−)/SD (−), the SM was not subjected to any incubation or solvent/detergent treatment. In the incubation (+)/SD (−) experiment, the pH of the SM was adjusted to 7.0, the solution was incubated at 30° C. for ten hours, and then the pH was reduced to 4.0. In the incubation (+)/SD, TNBP & Tween 80 (+) experiment, the pH of the SM was adjusted to 7.0, 3 mg/mL TNBP and 2 mg/mL Tween 80 were added to the solution, the solution was incubated at 30° C. for ten hours, and then the pH was reduced to 4.0. In the incubation (+)/SD, TNBP & cholate (+) experiment, the pH of the SM was adjusted to 7.0, 3 mg/mL TNBP and 2 mg/mL cholate were added to the solution, the solution was incubated at 30° C. for ten hours, and then the pH was reduced to 4.0. The solutions in each experiment were then diafiltered with four volumes CWFI (cold water for injection) and concentrated by ultrafiltration. After addition of dry maltose to 10% w/v, the 5% IGIV solution (pH 4.25) was filtered through a 0.2 μm filter.

TABLE 1

Anticomplement activity in 5% IGIV produced by variations of the Solvent/Detergent IGIV Process

	ACA (CH_{50}/mL)
Control (no solvent/detergent treatment, no 30° C. incubation)	25
Incubate at 30° C. for 10 hr (no solvent/detergent)	22
Incubate at 30° C. for 10 hr NLT 3 mg/mL TNBP NLT 2 mg/mL Tween 80	68
Incubate at 30° C. for 10 hr NLT 3 mg/mL TNBP NLT 2 mg/mL cholate	>100

*These samples were assayed for ACA after final compounding according to the Tenold '608 patent, but they were not incubated at pH 4.25 and 22° C. prior to analysis.

The results listed in Table 1 show that levels of ACA increased in IgG samples after incubation with TNBP/cholate or TNBP/Tween 80. ACA levels were not elevated in IgG samples that were incubated for 10 hr at 30° C. in the

absence of solvent/detergent. These results suggest that ACA levels of IGIV samples were not elevated by either processing manipulations or incubation for 10 hr at 30° C. in the absence of solvent/detergent.

TABLE 2

Anticomplement activity in 5% IGIV spiked with TNBP/Na cholate

	ACA (CH_{50}/mL)
5% IGIV, no TNBP/cholate	12
5% IGIV with 100 μg/mL TNBP, 100 μg/mL Na cholate	13

Furthermore, spiking experiments (with TNBP and Na cholate, Table 2) have demonstrated that the elevated anticomplement activity levels were not artifacts caused by carrying out the anticomplement assay in the presence of trace levels of TNBP/Na cholate. Thus, using the prior art SD process for viral inactivation of a solution containing ISG, subsequently formulated according to the Tenold '608 patent, yields a product which has high ACA and is unsuitable for intravenous administration. In a similar experiment, SD treated samples which were not incubated (Table 3, Initial Testing) had ACA levels greater than 100 units.

TABLE 3

Reduction in Anticomplement activity of samples previously treated with TNBP/cholate

Sample	ACA (CH_{50}/mL) Initial Testing (no incubation)	ACA (CH_{50}/mL) After incubation 6 wk. @ 5° C. 3 wk. @ 22° C.
RB21872-16	>100	33
RB21872-17	>100	34
RB21872-18	>100	36
RB21872-20	>100	27

However, when duplicate SD treated samples were incubated for extended periods of time (6 weeks at 5° C. and 3 weeks at 22° C.), the level of ACA was markedly reduced (Table 3, after incubation). This led to further investigation of this surprising observation.

Aggregate Content of ISG Exposed to TNBP/cholate

The samples of the previous experiment (Table 3, Initial Testing) were analyzed by size exclusion (gel permeation) HPLC immediately after compounding to determine the extent of aggregation of the IGIV at the initial time point. HPLC analysis shows nearly complete monomer content in the samples. (Table 4).

TABLE 4

HPLC analysis of non-incubated 5% IGIV samples (Table 3 Initial)

Sample	Aggregate (%)	Dimer (%)	Monomer (%)	Fragment (%)
RB21872-16, initial	0.140	0.00	99.86	0.00
RB21872-17, initial	0.146	0.00	99.85	0.00
RB21872-18, initial	0.124	0.00	99.88	0.00
RB21872-20, initial	0.172	0.00	99.83	0.00

Previously, high IgG aggregate levels were shown to correlate with high anticomplement activity. However, results from analysis of the samples show the level of ACA in the samples to be greater than 100 units. (Table 3, 'Initial Testing') The HPLC analysis shows that the high ACA following the TNBP/cholate treatment was not due to the presence of aggregated IgG molecules.

Varied Conditions of Time and Temperature

The SM was the same as in the previous experiment, and experimental conditions were similar with the following changes. The solutions were treated with TNBP/cholate at pH 7.0 and then were compounded to 5% IGIV, 10% maltose, pH 4.25, as above. The ACA was assayed immediately after final compounding, after a first incubation for nine days at 5° C., and after a second incubation for 21 days at either 22° C. or 5° C. The results are presented in Table 5.

TABLE 5

ACA of TNBP/cholate treated IGIV samples

Sample Point	ACA (CH_{50}/mL)
Intermediate Samples	
Initial sterile bulk	>100
Incubated 9 d. @ 5° C.	>100
Final Incubation	
21 d. @ 22° C.	49
21 d. @ 5° C.	71

In the initial sterile bulk sample, which was treated with TNBP/cholate at pH 7.0, the level of ACA was again greater than 100 units for the initial time point, confirming the observations noted in Table 3. Upon incubation at 5° C. for nine days, the ACA remained greater than 100 units. The final incubation step at either 5° C. or 22° C. shows that the reduction in ACA is dependent on temperature, with faster reduction in ACA observed at higher temperatures.

Effect of pH During Solvent/detergent Treatment on ACA

ACA levels were evaluated after incubation with TNBP/cholate at pH 5.8 because better viricidal activity was observed at pH values less than 6.0. Generally, the non-incubated sterile bulk samples of material incubated at pH 5.8 had lower ACA levels than the pH 7.0 samples, but the trend of lowering ACA upon incubation was repeated in the pH 5.8 samples. In fact, the ACA levels continue to decrease beyond the 21 day incubation in samples that initially had elevated ACA levels after incubation with TNBP/cholate at pH 5.8 (Table 6). As was previously noted for the samples incubated at pH 7.0, the lowering of ACA was not due to decreasing levels of aggregated IgG molecules because the material treated at pH 5.8 was essentially monomeric IgG prior to 22° C. incubation (HPLC analysis, sample A4, Table 8).

TABLE 6

Sample A4 - ACA upon extended incubation

Incubation at 22° C. (days)	CH_{50}/mL
0	122
10	73
19	55
25	56
28	45
30	40
34	39
41	33

TABLE 6-continued

Sample A4 - ACA upon extended incubation

Incubation at 22° C. (days)	CH_{50}/mL
48	30
55	29

Similar results were achieved with samples formulated to 10% IGIV, 0.2 M glycine in the sterile bulk stage. Upon incubation at low ionic strength at pH 4.25 for 10 and 21 days, the levels of ACA were seen to decline in both 5% IGIV samples and 10% IGIV samples. (Table 7) The decrease in ACA can thus be observed over a range of ISG concentrations and over a range of pH values for the solvent/detergent treatment. (Tables 3, 5, 7) HPLC analysis (Table 8) of the sterile bulk samples presented in Table 7 confirmed that the elevated ACA levels were not due to aggregation of ISG molecules.

TABLE 7

ACA of samples treated with TNBP/cholate at pH 5.8

Sample	Sterile bulk (day zero) (CH_{50}/mL)	10 days incubation at 20–27° C. (CH_{50}/mL)	21 days incubation at 20–27° C. (CH_{50}/mL)
A1 (5% IGIV)	43	ND	10
A2 (5% IGIV)	31	14	15
A3 (5% IGIV)	44	15	12
A4 (5% IGIV)	122	73	55
B1 (10% IGIV)	>100	48	46
B2 (10% IGIV)	49	36	30
B3 (10% IGIV)	53	ND	37

Taken together, the above results suggest that ISG products which have been subjected to a solvent/detergent viral inactivation process resulting in an undesirable ACA increase can be made suitable for IV administration by incorporating an additional incubation step under the conditions described here to reduce the ACA to an acceptable level.

TABLE 8

HPLC Analysis of sterile bulk samples treated with TNBP/cholate at pH 5.8

Sample	Aggregate (%)	Dimer (%)	Monomer (%)	Fragment (%)
A2	0.140	0.00	99.86	0.00
A3	0.146	0.00	99.85	0.00
A4	0.124	0.00	99.88	0.00

CONCLUSION

The ACA increase resulting from the solvent/detergent treatment of the IGIV (antibody) solution appears to be an unavoidable secondary effect of TNBP/detergent treatment to inactivate viruses in the solution. I have discovered that by incubating the solution of IGIV at low pH (4.25) and low ionic strength (0.001M) for a relatively long period of time (at least about 10 days), the ACA gradually decreases over the period of incubation.

The prior art discloses a method of producing IGIV (the Tenold '608 patent) using low pH and low ionic strength. The Tenold '608 method omits the viral inactivation step, and thus avoids the problem of increased ACA, but the possibility of viral activity remains. Unlike Tenold, incubation is an essential aspect of the present invention for reducing the ACA.

The Neurath et al. '573 patent teaches the solvent/detergent viral inactivation step. However, Neurath '573 does not mention controlling the pH and also does not mention any consequences of the process relating to ACA. Elevated ACA levels were detected at the sterile bulk stage of TNBP/cholate treated IGIV preparations. However, ACA levels decreased upon incubation for at least about 10 days at pH 4.25, low ionic strength, and not less than about 20° C. (See Tables 5–7) The prior art describes several approaches to lowering ACA levels of purified IgG preparations, including removal of IgG aggregates. (11) IgG aggregates have been shown to activate the complement system in vivo. (1) In the present invention, however, lowering of IgG ACA was not due to decreasing levels of IgG aggregates because these TNBP/cholate treated IGIV preparations contained low levels of aggregated IgG (as measured by HPLC, Tables 4, 8) prior to incubation under such conditions.

It would be desirable to produce substantially virus-free IGIV, but following the prior art results in a product with an unacceptable level of ACA. Note that Tenold '608 states that the product is substantially free of ACA, but use of the SD process in conjunction with Tenold '608 does result in high levels of ACA: experimental results reported here show that treating ISG solutions with the SD process and then formulation according to the Tenold '608 patent leads to a product with high ACA. (See Tables 1, 3, 5–7) The surprising finding reported here is that a follow-up (terminal) incubation step lowers the ACA of the solvent/detergent treated solution. The typical average observed ACA levels of 5% IGIV solutions treated according to the SD process and with or without the follow-up incubation are compared in the Figure. The present invention thus includes a previously unobserved method of reducing the ACA by incubating under controlled conditions of pH, temperature, and ionic strength for a period of time, thus allowing the product to be administered by intravenous injection.

Mitra '714 does not suggest the use of a S/D process but, instead, reports that a relatively brief incubation of an ISG product under similar conditions results in a substantially virus free preparation. (10) However, employing incubation under such conditions to provide a lowering of anticomplement activity is a novel application of these incubation conditions which were previously employed in the IGIV process for inactivation of enveloped viruses.

The newly developed IGIV process reported here, which includes an additional internationally accepted viral inactivation procedure (treatment with TNBP/cholate), generates IgG preparations which have low ACA levels and are suitable for IV administration. The major advantage is that an IGIV product with improved safety can be obtained by a two-step process that includes a TNBP/cholate treatment for viral inactivation and incubation under conditions that afford low ACA levels that are suitable for IV administration.

The above disclosure is intended to illustrate the invention, and it is thought variations will occur to those skilled in the art. Accordingly, it is intended that the scope of the invention should be limited only by the claims below.

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What is claimed is:

1. A method of treating a solution of antibodies which may have virus activity, the method comprising

a) contacting the solution with a trialkylphosphate and a detergent under conditions sufficient to substantially reduce any virus activity and resulting in an increased level of anticomplement activity; and

b) then incubating the solution of step a) under conditions of controlled time, pH, temperature, and ionic strength, such that the increased anticomplement activity of the solution is reduced to an acceptable level suitable for intravenous administration.

2. The method of claim **1**, wherein the anticomplement activity is reduced to less than about 60 CH_{50} units/mL.

3. The method of claim **1**, wherein the solution comprises about 5% wt./wt. antibody and the anticomplement activity is less than about 45 CH_{50} units/mL.

4. The method of claim **3**, wherein the solution comprises about 5% wt./wt. antibody and the anticomplement activity is less than about 30 CH_{50} units/mL.

5. The method of claim **1**, wherein the solution comprises about 10% wt./wt. antibody and the anticomplement activity is less than about 60 CH_{50} units/mL.

6. The method of claim **5**, wherein the solution comprises about 10% wt./wt. antibody and the anticomplement activity is less than about 45 CH_{50} units/mL.

7. The method of claim **1**, wherein the incubation is for at least about ten days.

8. The method of claim **1**, wherein the pH is maintained within a range of about 3.5 to about 5.0.

9. The method of claim **1**, wherein the temperature is maintained within a range of 2° C. to 50° C.

10. The method of claim **1**, wherein the ionic strength is less than about 0.001 M.

11. The method of claim **1**, wherein at least about 99% of the antibodies are monomeric.

12. The method of claim **1**, comprising the further step of adjusting the tonicity of the solution to a physiologic value under such conditions that the ionic strength is not appreciably altered.

13. The method of claim **12**, wherein the tonicity of the solution is adjusted by adding a carbohydrate to the solution.

14. The method of claim **13**, wherein the carbohydrate used is maltose.

15. The method of claim **12**, wherein the tonicity of the solution is adjusted to a range of about 230 to about 490 mosmol/kg solvent.

16. The method of claim **15**, wherein the tonicity of the solution is adjusted to a range of about 274 to about 309 mosmol/kg solvent.

17. The method of claim **12**, wherein the tonicity of the solution is adjusted by adding an amino acid to the solution.

18. The method of claim **17**, wherein the amino acid used is glycine.

19. The method of claim **1**, wherein the trialkylphosphate is tri-n-butyl phosphate and the detergent is selected from polysorbate 80 and sodium cholate.

20. The method of claim **1**, wherein the solution has a pH between about 3.5 and about 6.0 during step a).

21. An intravenously injectable immune serum globulin preparation produced by the method of claim **1** and substantially free of lipid enveloped viruses, wherein the preparation has an ionic strength less than about 0.001 M, a pH between about 3.5 and about 5.0, an antibody concentration of about 5% wt./wt., and a maltose concentration of about 10% wt./wt.

22. The preparation of claim **21**, wherein the pH is about 4.25.

23. An intravenously injectable immune serum globulin preparation produced by the method of claim **1** and substantially free of lipid enveloped viruses, wherein the preparation has an ionic strength less than about 0.001, a pH between about 3.5 and about 5.0, an antibody concentration of about 10% wt./wt., and a glycine concentration of about 0.2 M.

24. The preparation of claim **23**, wherein the pH is about 4.25.

* * * * *

EXHIBIT 2

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EXHIBIT 3

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EXHIBIT 4

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EXHIBIT 5

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EXHIBIT 6

EXHIBIT 6

Anticipation References

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5. Prince, *et al.*, "Failure of a human immunodeficiency virus (HIV) immune globulin to protect a chimpanzee against experimental challenge with HIV," Proc. Natl. Acad. Sci. USA 85:6944-6948 (1988) ("the Prince reference");
6. Biesert, *et al.*, "Virus validation of a new polyvalent intravenous immunoglobulin (Octagam)" *Vox Sang*. 67:S2 (1994); Abstract 0726, Octagam EU Brochure (1995); Octagam Israeli Brochure (1994) ("the Biesert/Octagam EU/Octagam Israeli reference");
7. Eriksson, *et al*. "Virus validation of plasma-derived products produced by Pharmacia, with particular reference to immunoglobulins," *Blood Coagulation and Fibrinolysis*, 5(3): S37-44 (1994) ("the Eriksson reference");
8. Venoglobulin-S product;

   Yang, Y.H. *et al.*, "Antibody Fc Functional Activity of Intravenous Immunoglobulin Preparations Treated with Solvent-Detergent for Virus

Inactivation," *Vox Sang*. 67:337-344;

Uemura, Y., *et al.*, "Inactivation and Elimination of Viruses during Preparation of Human Intravenous Immunoglobulin *Vox Sang.* 67:246-254 (1994) (together, "the Venoglobulin/Yang/Uemura reference");

9. Hämäläinen *et. al.*, "Virus Inactivation during Intravenous Immunoglobulin Production" *Vox Sang* 63:6-11 (1992) ("the Hämäläinen reference").

EXHIBIT 7

EXHIBIT 7

Obviousness References And Obviousness Combinations

1. U.S. Patent No. 5,256,771 (Tsay) (1993) ("the Tsay reference");
2. Paul K. Ng., *et al.*, "Process-Scale Purification of Immunoglobulin M Concentrate," Vox Sang 65:81-86 (1993) ("the Ng reference");
3. EP 525 502 A1 (Gehringer) (1992) ("the Gehringer reference");
4. Piet, *et al.*, "The use of tri(n-butyl) phosphate detergent mixtures to inactivate hepatitis viruses and human immunodeficiency virus in plasma and plasma's subsequent fractionation," Transfusion 30(7) 591-598 (1990) ("the Piet reference");
5. Prince, *et al.*, "Failure of a human immunodeficiency virus (HIV) immune globulin to protect a chimpanzee against experimental challenge with HIV," Proc. Natl. Acad. Sci. USA 85:6944-6948 (1988) ("the Prince reference");
6. Biesert, *et al.*, "Virus validation of a new polyvalent intravenous immunoglobulin (Octagam)" *Vox Sang*. 67:S2 (1994); Abstract 0726, Octagam EU Brochure (1995); Octagam Israeli Brochure (1994) ("the Biesert/Octagam EU/Octagam Israeli reference");
7. Eriksson, *et al*. "Virus validation of plasma-derived products produced by Pharmacia, with particular reference to immunoglobulins," *Blood Coagulation and Fibrinolysis*, 5(3): S37-44 (1994) ("the Eriksson reference");
8. Venoglobulin-S product;

   Yang, Y.H. *et al.*, "Antibody Fc Functional Activity of Intravenous Immunoglobulin Preparations Treated with Solvent-Detergent for Virus

Inactivation," *Vox Sang*. 67:337-344;

Uemura, Y., *et al.*, "Inactivation and Elimination of Viruses during Preparation of Human Intravenous Immunoglobulin *Vox Sang.* 67:246-254 (1994) (together, "the Venoglobulin/Yang/Uemura reference");

9. Hämäläinen *et. al.*, "Virus Inactivation during Intravenous Immunoglobulin Production" *Vox Sang* 63:6-11 (1992) ("the Hämäläinen reference");

10. U.S. Patent No. 4,396,608 (Tenold) ("the Tenold reference");

11. Barandun, S. *et al.*, Intravenous Administration of Human y-Globulin, *Vox Sang.* 7:157-174 (1962) ("the Barandun reference");

12. Wickerhauser, M. and Hao, Y., Large Scale Preparation of Macroglobulins, *Vox Sang*. 23:119-125 (1972) ("the Wickerhauser reference");

13. Louie, *et al.*, Inactivation of Hepatitis C Virus in Low pH Intravenous Immunoglobulin, Biologicals 22:13-10 (1994) ("the Louie reference").

Baxter contends that Claims 1, 8 through 10, 12, and 15 through 20 are obvious in view of at least:

1. The Tsay reference in combination with the Ng reference;

2. The Gehringer reference in combination with the Louie reference and/or the Ng reference;

3. The Ng reference in combination with the Wickerhauser reference and/or the Tsay reference;

4. The Prince reference in combination with the Tsay reference and/or the Tenold reference;

5. The Piet reference in combination with the Tsay reference, the Wickerhauser reference and/or the Louie reference;

6. The Eriksson reference in combination with the Tsay reference, the Ng reference and/or the Barandun reference;

7. The Biesert/Octagam EU/Octagam Israeli reference in combination with the Barandun reference, the Louie reference, the Tenold reference and/or the Hämäläinen reference;

8. The Venoglobulin/Yang/Uemura reference in combination with the Barandun reference; and/or

9. The Hämäläinen reference in combination with the Louie reference and/or the Tenold reference.

Baxter also contends that Claim 7 is obvious in view of at least:

1. The Tsay reference in combination with the Ng reference, the Louie reference, the Hämäläinen reference and/or the Barandun reference;

2. The Gehringer reference in combination with the Ng reference, the Louie reference and/or the Barandun reference;

3. The Ng reference in combination with the Louie reference and/or the Barandun reference;

4. The Prince reference in combination with the Louie reference and/or the Barandun reference;

5. The Piet reference in combination with the Louie reference and/or the Barandun reference;

6. The Eriksson reference in combination with the Louie reference and/or the Barandun reference;

7. The Biesert/Octagam EU/Octagam Israeli reference in combination with the Louie reference and/or the Barandun reference; and/or

8. The Venoglobulin/Yang/Uemura reference in combination with the Louie reference and/or the Barandun reference.

EXHIBIT 8

THIS EXHIBIT HAS BEEN REDACTED IN ITS ENTIRETY

IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

CERTIFICATE OF SERVICE

I, Philip A. Rovner, hereby certify that on May 21, 2007, the within document was filed with the Clerk of the Court using CM/ECF which will send notification of such filing(s) to the following; that the document was served on the following counsel as indicated; and that the document is available for viewing and downloading from CM/ECF.

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